CHLOROPHACINONE AND DIPHACINONE: STANDARD *MUS MUSCULUS* AND *PEROMYSCUS MANICULATUS* ANTICOAGULANT LABORATORY TESTS

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ABSTRACT: The Vertebrate Pest Control Research Advisory Committee, through a cooperative agreement with the California Department of Food and Agriculture (CDFA), funded laboratory studies at the National Wildlife Research Center (NWRC). The objective of the studies was to obtain efficacy data for controlling house mice (*Mus musculus*) and deer mice (*Peromyscus maniculatus)* that would provide partial fulfillment of the requirements established by the Environmental Protection Agency (EPA) for the re-registration of the CDFA's 0.01% chlorophacinone and 0.01% diphacinone grain bait labels. Swiss-Webster mice and deer mice from an on site breeding colony were placed on 15-day, two-choice feeding and efficacy trials. The control treatment groups received two dishes each containing the Office of Pesticide Programs (OPP) designated standard rat and mouse challenge diet. The treated groups received one dish of the standard OPP rat and mouse challenge diet and a second dish of the treated grain baits: 0.01% chlorophacinone (two groups of 20 animals, Groups II and III), or 0.01% diphacinone (two groups of 20 animals, Groups II and III) grain bait. Results from the treated groups of the four tests are reported. House mice on 0.01% chlorophacinone: female mortality was 100% in Group II and 90% in Group III. Total dietary consumption of treated bait was 23.0% of the total bait consumption. House mice on 0.01% diphacinone: female mortality was 90% in Group II and 100% in Group III. Total dietary consumption of treated bait was 18.2% of the total bait consumption. Deer mice on 0.01% chlorophacinone: female mortality in Groups II and III was 100%. Total dietary consumption of treated bait was 63.1% of the total bait consumption. Deer mice on 0.01% diphacinone: female mortality in Groups II and III was 100%. Total dietary consumption of treated bait was 66.3% of the total bait consumption. In conclusion, the female house mice ate less than the male house mice with the same mortality rate (between 90% and 100%); while the female *Peromyscus* ate more than the male *Peromyscus,* they had the same mortality rate (100%). The anticoagulant grain bait test mortality results for both house mice and deer mice met the suggested performance standard for the EPA Pesticide Assessment Guidelines.

KEY WORDS: *Mus musculus*, *Peromyscus maniculatus*, house mice, deer mice, anticoagulants

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INTRODUCTION

In 1993, the Environmental Protection Agency (EPA) suggested to the California Department of Food and Agriculture (CDFA) that efficacy data would be required for CDFA's 0.01% chlorophacinone and 0.01 diphacinone oat groat bait registrations for controlling house mice (*Mus musculus*) and deer mice (*Peromyscus* spp.). To meet this objective, the Vertebrate Pest Control Research Advisory Council (VPCRAC) through a cooperative agreement from the CDFA, funded the National Wildlife Research Center (NWRC) to conduct laboratory feeding trials that evaluated mortality and bait acceptance among mice fed an oat groat bait formulated with technical chlorophacinone or diphacinone. The feeding trials were conducted in compliance with Code of Federal Regulations (CFR) 40, Part 160, Good Laboratory Practices (Sirofchuck 1996) and the appropriate Pesticide Assessment Guidelines (PAG), Subdivision G (U.S. EPA 1982). The test methods conformed to the "Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method" and the "Standard *Peromyscus* Species Anticoagulant Dry Bait Laboratory Test Method" as outlined in Office of Pesticide Programs (OPP) Designation 1.204 (2-25-74) and 1.216 (2-25-74), respectively (McCann 1980 a and b). The total amount of OPP rat and mouse challenge diet and treated bait consumed over the 15-day feeding trial was used to determine bait acceptance. Dietary consumption of the treated bait must account for 33% or greater of the total diet to meet EPA standards. The EPA also requires a minimum of 90% mortality for laboratory tests done with anticoagulants.

MATERIALS AND METHODS

Bait Formulation

The 0.01% chlorophacinone oat groat baits and the 0.01% diphacinone oat groat baits were formulated by Rodent Control Outfitters, Inc.[1,2], and were prepared according to the Confidential Statement of Formula for CDFA registrations. The 0.01% chlorophacinone grain baits and the 0.01% diphacinone oat groat baits were assayed by the Analytical Chemistry Unit of the NWRC using validated method 62A.

OPP Rat and Mouse Challenge Diet

The OPP rat and mouse challenge diet was prepared at the NWRC as specified by the EPA. It was formulated according to the instructions in OPP Designations (1.204

[1]Reference to commercial products or entities does not imply endorsement by U.S. Government.

[2]Mailing address: P.O. Box 446, Junction City, OR 97448.

and 1.216) (McCann 1980 a and b). The ingredients are as follows:

Ingredients	% by Weight
Cornmeal (whole yellow ground corn)	65%
Rolled Oat Groats (ground)	25%
Sugar (powdered ≥95% purity)	5%
Corn Oil (≥95% purity)	5%
Total	100%

After formulation, the OPP rat and mouse challenge diet was packaged in plastic containers, tightly sealed, and maintained at -18°C or colder until used. Before being offered to test or control mice, the OPP rat and mouse challenge diet was brought to room temperature.

TEST PROCEDURES

Animal Procurement

A total of 136 sexually mature house mice (*Mus musculus*) of the Swiss-Webster genotype were purchased at two different times from Simonsen Laboratories, Inc.[3,4] for the chlorophacinone and diphacinone feeding studies. Sixty-eight house mice (34 males and 34 females) for the chlorophacinone study weighed between 19.1 g and 31.5 g; and the 68 (34 males and 34 females) house mice attained for the diphacinone study weighed between 14.3 g and 18.8 g.

As a population of deer mice (*Peromyscus maniculatus*) would be needed for feeding trials at NWRC, a breeding colony of deer mice was established in late November 1997, at the NWRC's Animal Research Building (ARB). Ten males and ten females weighing 15 to 16 g each were purchased (certified hantavirus disease free) from the University of South Carolina, Columbia, South Carolina.

Quarantine

After their arrival on November 19, 1997, at the NWRC's ARB, each deer mouse was weighed and then placed in an individual cage (27.9 x 17.8 x 12.7 cm). The mice were maintained on a commercial laboratory mouse diet and provided water *ad libitum* for seven days. Following completion of the seven-day quarantine period, the deer mice were checked by the resident veterinarian and released to start the breeding colony.

The house mice underwent the same seven-day quarantine as the initial 20 deer mice and the room temperature was maintained between 19°C and 22°C, while the light cycle was 12 hours light and 12 hours dark for both species.

Breeding Colony

The deer mouse breeding colony remained in the same room used during the quarantine period. The ten male deer mice and ten female deer mice were paired on November 26, 1997, and the breeding colony was maintained until enough offspring were produced to conduct the required laboratory tests.

Assignment of Treatments

The four feeding trials were run separately. All house mice and deer mice (eligible sexually mature offspring) were weighed and individually placed in numbered single-sized cages (27.9 x 17.8 x 12.7 cm). Eight house mice and six deer mice were randomly selected from the total available population (68 house mice and 66 deer mice) and eliminated from further testing. The remaining 60 mice (30 males, 30 females) were ranked by weight into ten weight classes, each containing three animals, sexes separate. Each weight class for both genders contributed one animal to each of the three groups (I, II, and III). The six groups of ten mice, separated by gender were randomly assigned to one of the following groups: 0.0% control (Group I); 0.01% chlorophacinone bait or 0.01% diphacinone bait (Group II); and 0.01% chlorophacinone bait or 0.01% diphacinone (Group III). This grouping resulted in ten mice per gender per group, or a total of 20 animals per group, and evenly distributed the mice by body weights among the three groups.

Three-Day Acclimation Period

After being grouped and placed into their assigned triple-sized cages (63.5 x 24.1 x 17.8 cm), the mice were acclimated to their new groups for three days. Each of the six cages was labeled by sex and treatment, and the test room temperature was maintained between 19° and 22°C, under the same light cycle of 12 hours light and 12 hours dark for both species.

The 15-Day Test

Day 1. On the first day of testing, the regular diet was removed from all cages. Each group was then offered two rectangular aluminum dishes (15.24 x 3.81 x 3.81 cm), located on opposite sides of the cage. Group I (control) received two dishes (one labeled O and one labeled C) each containing (in excess of 40 g) OPP rat and mouse challenge diet. Mice in Groups II and III received the 0.01% chlorophacinone or the 0.01% diphacinone grain bait, and were given one dish containing (in excess of 40 g) treated bait (labeled T) and a second dish containing (in excess of 40 g) OPP rat and mouse challenge diet (labeled O). Trays were placed beneath each cage to catch treated bait and OPP rat and mouse challenge diet spillage. Dishes containing the treated bait were positioned on the left, and dishes containing the OPP rat and mouse challenge diet were placed on the right, both at the front of the cage. Bottled water was available to the animals at all times and was positioned at equal distances from the bait dishes also at the front of the cage. The animal room was closed until Day 2 except for minimal and unobtrusive observation, as conditions permitted, to monitor the animals. The 24-hour period from the time the bait was issued until its removal the next morning was considered Day 1.

Day 2. All bait dishes and any spillage were removed and re-weighed to determine the gross weight to the nearest 0.01 g. The amount of bait consumed was

[3]Reference to commercial products does not imply endorsement by U.S. Government.
[4]Mailing address: 1180-C Day Road, Gilroy, CA 95020.

recorded. Sometimes the bait weighed more than it did initially and may have gained moisture either from humidity or urine in the dishes. Those particular bait weights were reported as a gain and omitted when summing the total amount of bait consumed. Dishes were replenished with fresh bait to bring them back to their original weight, while any bait that was fouled with urine or feces was discarded. Dish positions were reversed when returned to the cages (i.e., treated bait dishes on the right, OPP rat and mouse challenge diet dishes on the left). Any mortalities were removed and weighed. The animal room was then closed as described for Day 1.

Days 3-14. Days 3-14 were a repeat of Day 2, except the positions of the dishes were alternated each day. Any carcasses were removed and weighed.

Day 15. On Day 15, the animals were checked for mortality and the baits, including bait spillage, were removed and weighed.

Five-Day Post-Testing Feeding Period

Day 16. All survivors were then placed on the OPP rat and mouse challenge diet for five days. All control animals and groups of surviving mice were offered one rectangular metal dish containing (in excess of 40 g) the OPP rat and mouse challenge diet. The position of the dish (labeled O) was alternated each day. Trays were placed beneath each cage to catch spillage. Bottled water was available at all times and was positioned in the center of the front of the cages. The animal room was closed until Day 16 except for minimal and unobtrusive observations, as conditions permitted, to monitor the animals.

Days 17-19. The OPP rat and mouse challenge diet, including any bait spillage, was again removed and weighed, following the procedure outlined for Day 16 (five-day post-testing feeding period). Animals were checked for mortality.

Day 20. The bait and any spillage were removed and weighed. Survivors were weighed and euthanatized with CO_2 and frozen until the carcasses could be incinerated.

Data Recorded and Statistics

Daily consumption for both bait dishes for each group of mice, body weights of mice, dates of mortality, or euthanasia were recorded: 1) pre-treatment; 2) after death; or 3) at the end of the study. Bait consumption and body weights were totaled for each group, and a mean and standard deviation (SD) were computed.

RESULTS

Bait Analysis

The 0.01% chlorophacinone bait for house mice was assayed at a mean of 0.0108%; the 0.01% diphacinone bait for the house mice was assayed at a mean of 0.0116%; the 0.01% chlorophacinone bait for the deer mice was assayed at a mean of 0.0098%; and the 0.01% diphacinone bait for the deer mice was assayed at a mean of 0.015%.

Mortality and Bait Consumption

House mice on 0.01% chlorophacinone. Female mortality was 100% in Group II and 90% in Group III (Table 1). Total treated bait consumption for the 15-day feeding trial for female groups was 28.17 g and 23.13 g, respectively (Table 2). Male mortality was 90% in Group II and 100% in Group III (Table 1). Total treated bait consumption for the 15-day feeding trial for male groups was 42.26 g and 40.19 g, respectively. Mice began to die on Day 3, and the total dietary consumption of treated bait was 23.0% of the total bait consumption (Table 2).

House mice on 0.01% diphacinone. Female mortality was 90% in Group II and 100% in Group III (Table 1). The total treated bait consumption for the 15-day feeding trial for female groups was 20.22 g and 23.18 g, respectively (Table 2). Male mortality was 90% in Group and 100% in Group III (Table 1). The total treated bait consumption for the 15-day feeding trial for male groups was 23.18 g and 28.40 g, respectively. Mice began to die on Day 3 and the total dietary consumption of treated bait was 18.2% of the total bait consumption (Table 2).

Deer mice on 0.01% chlorophacinone. Female mortality in Groups II and III was 100% (Table 1). The total treated bait consumption for the 15-day feeding trial for female groups was 43.61 g and 75.27 g, respectively (Table 2). Male mortality in Groups II and III was 100%, also (Table 1). The total treated bait consumption for the 15-day feeding trial for male groups was 23.02 g and 49.27 g, respectively. Mice began to die on Day 1 and the total dietary consumption of treated bait was 63.1% of the total bait consumption (Table 2).

Deer mice on 0.01% diphacinone. Female mortality in Groups II and III was 100% (Table 1). The total treated bait consumption for the 15-day feeding trial for female groups was 61.63 g and 77.97 g, respectively (Table 2). Male mortality in Groups II and III was 100%, also (Table 1). The total treated bait consumption for the 15-day feeding trial for male groups was 45.56 g and 85.44 g, respectively. Mice began to die on Day 3 and the total dietary consumption of treated bait was 66.3% of the total bait consumption (Table 2).

DISCUSSION

In both house mouse trials, the mice preferred the OPP rat and mouse challenge diet over the 0.01% treated bait. Their consumption of the treated bait never exceeded that of the OPP rat and mouse challenge diet during the test. Despite the low toxic bait consumption, the 40 treated mice in each test ingested enough to cause sufficient mortality to meet and exceed the 90% minimum standard for mortality established by the EPA for verifying efficacy of rodenticides. Consumption for the house mice that survived the 0.01% chlorophacinone test was not measurable until it was the last animal in the cage. The male survivor consumed 7.02 mg/kg and the female consumed 10.08 mg/kg over a period of seven days. Jackson and Ashton (1992) found LD_{50} values for house mice to be 0.42 mg/kg for males and 2.83 mg/kg for females. Without knowing the slope of the line when plotting the LD values, it cannot be determined if the survivors consumption exceeded the LD_{99} value. Rowe and Redfern (1968) measured mortality and bait acceptance among house mice given both chlorophacinone and diphacinone oat baits. Four no-choice tests were conducted, each one lasting either 3, 7, 14, or 18 days.

Table 1. Percent mortality in treated groups of mice.

Treatment	Sex	Group II	Group III
House mice on chlorophacinone	F	100	90
	M	90	100
House mice on diphacinone	F	90	100
	M	90	100
Deer mice on chlorophacinone	F	100	100
	M	100	100
Deer mice on diphacinone	F	100	100
	M	100	100

The concentrations tested were: chlorophacinone 0.005% and 0.025%, and diphacinone 0.0125% and 0.025%. Mortality for the 3-day test was less than 50% on all four concentrations. For the 7-day test, 100% mortality occurred at 0.025% chlorophacinone concentration, and 50 to 80% mortality occurred at the three other concentrations. For the 14-day test, 100% mortality occurred again at 0.025% chlorophacinone concentration, and the mortality for the other three concentrations increased to 80% to 90%. For the 18-day test, only the 0.0125% diphacinone concentration failed to achieve 100% mortality. At this concentration, two of their mice survived and consumed a total of 255 and 466 mg/kg of diphacinone. Under laboratory conditions, Lund (1971) fed 0.025% chlorophacinone oat groats bait to groups of 20 mice for each test. His tests consisted of an array of feeding periods varying from 1 to 21 days. When the feeding period was 1 to 5 days, mouse mortality was 5% to 75%; whereas, when the feeding period was increased to 6, 10, and 21 days, mortality was 80%, 90%, and 95%, respectively. He fed one house mouse as much as 906 mg/kg and it survived.

In both of this study's deer mouse trials, the mice preferred the treated bait over the OPP rat and mouse challenge diet and consumed slightly more diphacinone than chlorophacinone. Marsh et al. (1977) suggest that there may be a "greater degree of susceptibility by deer mice to chlorophacinone" and that "it has the potential to surpass the degree of deer mouse control previously experienced with diphacinone." House mice and deer mice show occasional individual variation in consumption and anticoagulant affect (Lund 1971 and Howard et al. 1970).

To summarize the findings of the four tests:

House mice on 0.01% chlorophacinone. Total dietary consumption of treated bait was 23% of the total bait intake and overall mortality was 95%.

House mice on 0.01% diphacinone. Total dietary consumption of treated bait was 18.2% of the total bait intake and overall mortality was 95%.

Deer mice on 0.01% chlorophacinone. Total dietary consumption of treated bait was 63% of the total bait intake and overall mortality was 100%.

Deer mice on 0.01% diphacinone. Total dietary consumption of treated bait was 66.3% of the total bait intake and overall mortality was 100%.

All four tests exceeded the 90% minimum standard established by the EPA for verifying the efficacy of rodenticides.

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Table 2. Amount and proportion of treated bait consumed compared to OPP rat and mouse challenge diet.

Treatment, Sex, and Group	Total Bait Consumption OPP (g)	0.01% (g)	Total (g)	Percent Treated Bait in Total Diet
House mice on 0.01% chlorophacinone				
Females				
Group II	82.22	28.17	110.39	25.52
Group III	140.82	23.13	163.95	14.11
Males				
Group II	120.58	42.26	162.84	25.95
Group III	103.39	40.19	143.58	27.99
Total	447.01	133.75	580.76	23.03
Proportion of treated bait in diet = $\frac{133.75\ g}{580.76\ g}$ = 23.0%				
House mice on 0.01% diphacinone				
Females				
Group II	91.70	20.22	111.92	18.07
Group III	100.09	23.18	123.27	18.80
Males				
Group II	139.13	23.18	162.31	14.28
Group III	95.81	28.40	124.21	22.86
Total	426.73	94.98	521.71	18.21
Proportion of treated bait in diet = $\frac{94.98\ g}{521.71\ g}$ = 18.2%				
Deer mice on 0.01% chlorophacinone				
Females				
Group II	36.36	43.61	79.97	54.53
Group III	16.32	75.27	91.59	82.18
Males				
Group II	39.60	23.02	62.62	36.76
Group III	19.55	49.27	68.82	71.59
Total	111.83	191.17	303.00	63.09
Proportion of treated bait in diet = $\frac{191.17\ g}{303.00\ g}$ = 63.1%				
Deer mice on 0.01% diphacinone				
Females				
Group II	35.23	61.63	96.86	63.63
Group III	19.87	77.97	97.84	79.69
Males				
Group II	51.94	45.56	97.50	46.72
Group III	30.71	85.44	116.15	73.56
Total	137.75	270.60	408.35	66.27
Proportion of treated bait in diet = $\frac{270.60\ g}{408.35\ g}$ = 66.3%				